

Research Article



Investigation the Existence of H4 Gene in Leishmania Tropica

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ABSTRACT

Cutaneous Leishmaniasis caused by *Leishmania tropica* is one of the most important health issues in Syria which exacerbated after what happening in Syria. Prevention from Leishmaniasis is very important to reduce the high prevalence of infection in Syria. Genetic vaccination has recently provided a promising new approach to vaccination. Nucleosomal core histones are very important for the parasites life so we choose one of these genes, H4 gene, to design a DNA vaccine against the infection with *L. tropica*. Our study aimed to investigate the existence of H4 gene in a Syrian strain of *L. tropica* promastigotes and their expression. We isolated DNA and RNA from these parasites, synthesized cDNA, design the necessary primers for gene's amplification, and standardized the PCR conditions. We proved existence of this gene in *L. tropica* promastigotes. Its size was 303 bp. Expression was also proved in these parasites. The sequence of the cDNA H4 gene was defined in a Syrian strain *L. tropica*.

Keywords: *Leishmania tropica*, H4 gene, Syria.

INTRODUCTION

Leishmaniasis are infectious diseases caused by several species of the intracellular protozoan of the genus *Leishmania*, which are transmitted by the bite of an infected female phlebotomine sandfly¹. The different clinical manifestations of leishmaniasis, ranging from self-healing cutaneous ulcers to potentially fatal visceral infection, are determined by the species of *Leishmania* that infects the host and by the response of the host to the parasite¹. These diseases represent a global public health problem, with an estimated prevalence of 12 million human beings infected. The majority of cutaneous Leishmaniasis cases occur in Afghanistan, Algeria, Brazil, Colombia, the Islamic Republic of Iran, Pakistan, Peru, Saudi Arabia and the Syrian Arab Republic². Current control is based on chemotherapeutic treatments which are expensive, toxic and associated with high relapse and resistance rates. Vaccination remains the best hope for control of all forms of the disease, so the development of a safe, effective and affordable antileishmanial vaccine is a critical global public-health priority. Immunization with naked DNA is a relatively new approach, which promises to revolutionize the prevention and treatment of infectious diseases.³ Many studies have been made to find a protective antigen against leishmaniasis and many candidates have been studied for this aim, including histones. Histones are structural proteins found in the nucleus, where they play an important role in the organization and function of chromatin⁴. These highly-conserved proteins that elicit strong immune responses are generally designated as panantigens⁵. Specific antibodies for parasite histones, obtained from dogs with visceral leishmaniasis, react against *Leishmania* H2A⁶, H3⁷, H2B and H4⁸ but do not recognize mammalian histones. The capacity of

panantigens to modulate the immune system can be related to the fact that these intracellular proteins were not selected by the immune pressure, unlike the secreted and surface proteins. Hence, in the right conditions, they can provide the immunomodulatory properties needed for vaccine design⁹. For these reasons we choose one of these genes, H4 gene, to design a DNA vaccine against the infection with *Leishmania tropica* in Syria. A methodical study was first made to know if this gene exists in the genome of a Syrian strain of *Leishmania tropica*, to prove if there is an expression of H4 Gene in these parasites and to sequence this gene.

MATERIALS AND METHODS

Primer design for PCR

Sequence of H4 gene in *Leishmania tropica* is not known so we made an alignment for the sequences of this gene in other species using CLC free workbench 7 and Vector NTI Express programs and the clustalw2 and emboss_needle tools. Primers were designed using <http://www.idtdna.com/calc/analyzer> and http://www.geneinfinity.org/sms/sms_primanalysis.html tools. High purified Primers were ordered from VBC-Biotech, Vienna. Final sequences for primers were: (forward, 5'-ATCA GAATTCATG GCC AAG GGC AAG CGC TC-3'; reverse, 5'-ATGTCTAGA TTACGCGTAGCCGTACAGGATG-3'). Underlined are the ECORI and XBAI restriction sites included for direct cloning in the PCI mammalian expression vector (Promega, United States).

DNA Extraction

DNA was extracted from the promastigotes of a Syrian strain *Leishmania tropica* (LCEBS, Damascus, Syria). DNA was isolated from *Leishmania* promastigotes, cultured on



RPMI-1640 medium (Lonza, Switzerland) supported with Fetal bovine serum (Cytogen, GmbH, Germany), with DNA extraction kit (Thermoscientific, Lithuania) according to manufacturer's instructions.

RNA Isolation and cDNA Synthesis

Total RNA was extracted from harvested *Leishmania tropica* promastigotes using TRI reagent (Sigma, St Louis, MO, USA). RNA was quantified by NanoDrop1000 (Thermo Fisher Scientific MA, USA). RNA-integrity was checked by separating in 1.5% agarose gel. First strand cDNAs were synthesized using RevertAid First Strand cDNA Synthesis kit (Thermoscientific, Lithuania) according to manufacturer's instructions.

Polymerase Chain Reaction

DNA and cDNA of H4 gene were amplified by thermal cycler (Bio-Rad, USA) using PCR master mix (Thermoscientific, Lithuania) and the primers mentioned above. PCR protocol was optimized using gradient annealing temperatures. Reactions were performed in 25 μ l of a mixture containing 1 μ l of 10 μ M from each primer, 2,5 μ l of template DNA or cDNA (110 ng μ l), 12,5 μ l of PCR master mix and 8 μ l of PCR water. Thermal cycling conditions were 95°C for 5min followed by 35 cycles of 95°C for 1min, 62°C for 45sec and 72°C for 1 min finally 72°C for 5 min. PCR products were loaded on a 2% agarose gel, stained with ethidium bromide, and visualized on a UV transilluminator.

Plasmid construction and purification

PCR product containing H4 coding sequence was purified using Gene Jet PCR Purification kit (Thermoscientific, Lithuania) according to manufacturer's instructions. Purified cDNA and the PCI mammalian expression vector (Promega, United States) were double digested with ECORI plus XBAI. Digested cDNA was ligated with the digested PCI mammalian expression vector using t4 DNA ligase (Thermoscientific, Lithuania). PCI-H4 clones were transformed in to Ecoli TOP 10. Recombinant plasmid was extracted from colonies and purified using GF-1 plasmid DNA extraction kit (Vivantis, Malaysia).

Sequences Analysis

Full-length double-stranded sequence analysis was performed on purified Recombinant plasmid PCI-H4 by sequencing, using T7 (forward) and PCR 3' (reverse) vector primers on automated sequencers (ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit, Perkin-Elmer, Foster City, USA).

RESULTS AND DISCUSSION

Evaluation DNA and RNA quality and purity

Extracted DNA electrophoresis on a 1% agarose gel showed only one band which proves that there isn't any degradation in the extracted DNA (figure 1). The purity of the DNA was 1.8 which showed a high degree of purity. Extracted RNA electrophoresis on a 1.5% agarose gel

showed a standard profile and there was no contamination with genomic DNA (figure 2). The purity of the RNA was 2.1 which showed a sufficient degree of purity.

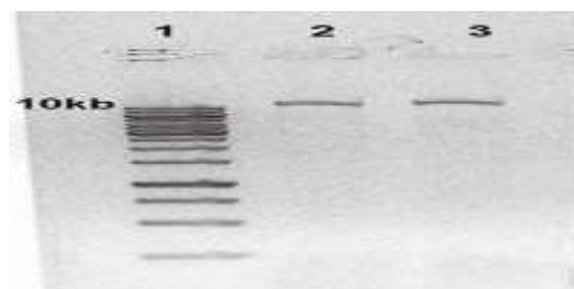


Figure 1: Electrophoresis of extracted *Leishmania tropica* DNA: Lane 1 DNA ladder 1Kb, Lane 2,3 extracted *Leishmania tropica* DNA.

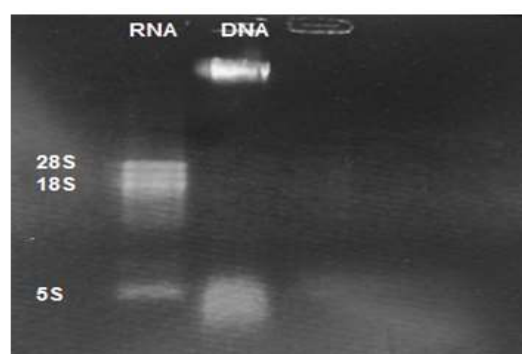


Figure 2: Electrophoresis of extracted *Leishmania tropica* RNA: Lane1 *Leishmania tropica* RNA, Lane 2 extracted *Leishmania tropica* DNA.

Polymerase Chain Reaction

PCR protocol was optimized using gradient annealing temperatures and we chose the most suitable annealing temperature which was 62°C. We don't compare our result with other studies since they didn't mention their PCR conditions^{10,11} Gel electrophoresis of H4 PCR product on the level of DNA and RNA showed only one band of 303 base pair which proves the specificity of our primers. So we proved the existence of H4 gene in the genome of a Syrian strain of *Leishmania tropica* and in the RNA of this strain (figure 3).



Figure 3: Amplification products of *Leishmania tropica* H4 on 2% agarose gel electrophoresis stained with ethidium bromide. Lane 1 DNA ladder 50bp, lane 2, 3 H4-DNA

amplification product of 303 bp, lane 4 H4-cDNA amplification product of 303 bp.

Cloning and sequencing of the H4 cDNA

In an effort to clone and characterize the Leishmania tropica H4 gene, a cDNA clone was identified and sequenced. The sequence was submitted to the Gene bank under KT956067 Accession number. Figure 3 shows the complete nucleotide sequence of the 303bp cDNA insert and the deduced amino acid sequence in comparison with other species. The deduced amino acid sequence predicts a protein containing 100 amino acids

and having a predicted molecular mass of 11,439 Da. The H4 predicted amino acid sequence was compared with the sequences of proteins coded by cDNA of H4 in other species of Leishmania such as L.infantum, L.major, L.donovania, L.mexicana and L.braziliensis using Vector NTI Program. This search revealed that H4 belongs to the histone H4 family.

Interestingly, L.tropica H4 shows significant similarity with H4 histones of L.infantum, L.major, L.donovania (96%), L.mexicana and L.braziliensis (91%).

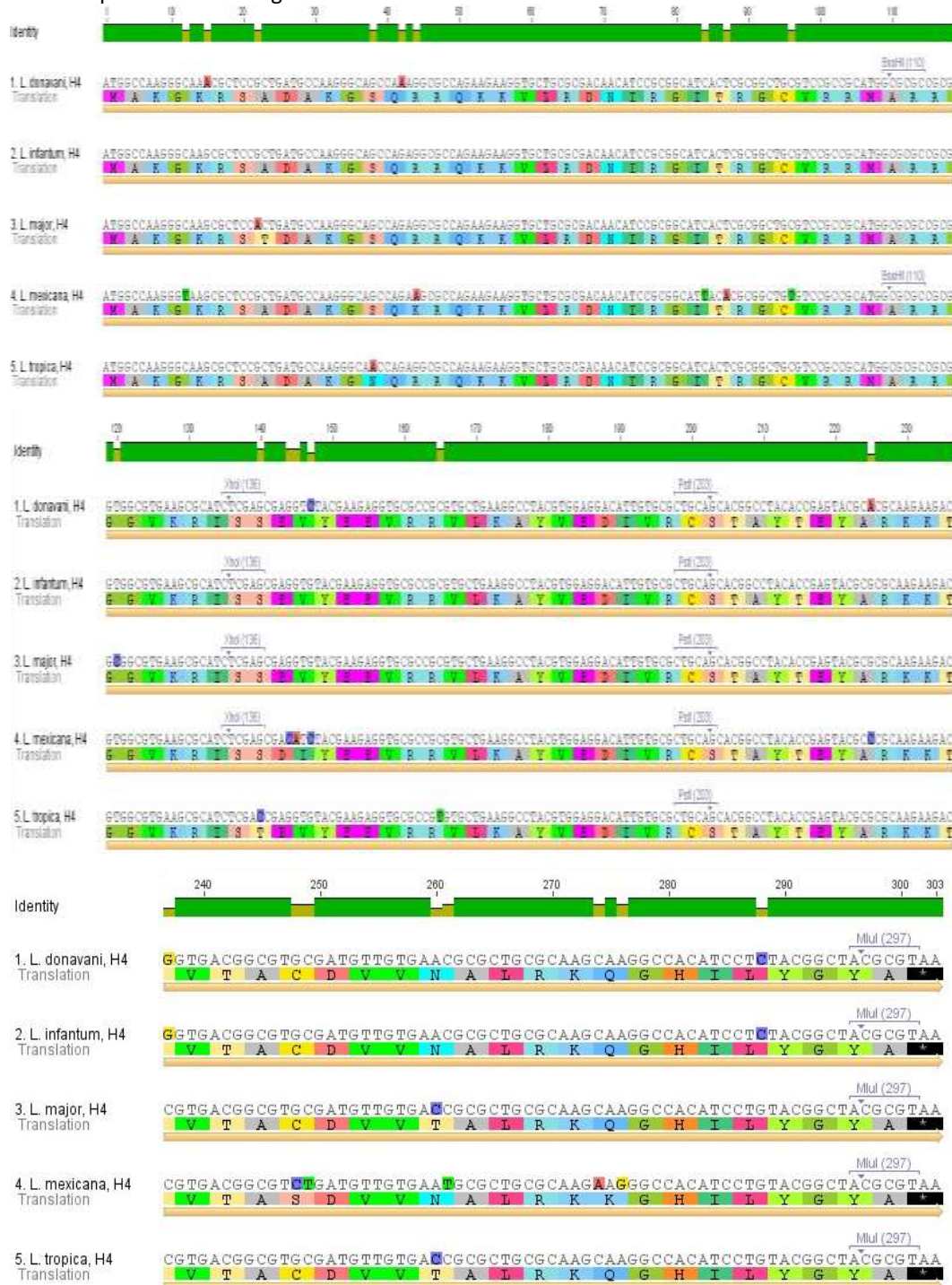


Figure 4: Nucleotide sequence of L. tropica H4 cDNA. The deduced amino acid sequence is indicated below the cDNA sequence.

CONCLUSION

Our results proved the existence of H4 gene in the genome of Syrian strain *Leishmania tropica* and in the RNA of this strain.

We defined the sequence of the cDNA of *Leishmania tropica* H4 gene and submitted it to the Gene bank under KT956067 accession number.

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